Figure 1

Nucleotide sequence of the *Prunus amygdalus HNL5* gene obtained by PCR amplification

The start codon (ATG) and stop codon of the open reading frame are printed in bold type, and the nucleotides in the intron regions are indicated in lower case letters. The peripheral sequences which have been attached via the PCR primers and which are not part of the *HNL5* gene are underlined. The splice sites of the introns were identified with the aid of the consensus sequence "GT....AG".

1 GGAATTCACA ATATGGAGAA ATCAACAATG TCAGTTATAC TATTTGTGTT

51 GCATCTTCTT GTTCTTCATC TTCAGTATTC AGAGGTTCAC TCGCTTGCCA

101 ATACTTCTGC TCATGgtaaa tttccatctt cagtattcat ttaacagcaa

151 aatgtgtaga tttataatta agaaaactga cacaagtagt gcaagaaaca

201 agctaattta gatgcatgtt gaaaaaaatc tttcatctct tcacatatat

251 tttgcagATT TTAGCTACTT GAAGTTTGTG TACAACGCCA CTGATACAAG

301 CTCGGAAGGA TCATATGACT ACATTGTAAT CGGTGGAGGA ACATCAGGGT

351 GTCCATTGGC AGCAACTTTA TCAGAAAAAT ACAAGGTGCT TCTTCTAGAA

401 AGAGGCACTA TTGCTACAGA ATACCCGAAC ACGTTGACTG CAGATGGGTT

451 TGCATATAAT CTGCAGCAAC AAGATGATGG AAAGACGCCA GTTGAAAGGT

501 TCGTGTCCGA AGATGGCATT GATAATGTGC GAGCCAGGAT CCTCGGTGGC

551 ACGACCATAA TCAATGCAGG CGTCTACGCC AGAGCTAACA TTTCATTCTA

601 TAGTCAAACA GGAATTGAAT GGGACCTGGA TTTGGTCAAT AAGACATATG

651 AGTGGGTTGA AGACGCCATT GTGGTCAAGC CAAATAATCA ATCTTGGCAA

701 TCTGTTATAG GAGAGGGATT CTTGGAGGCG GGTATTCTTC CAGACAATGG

751 ATTTAGTTTG GATCACGAAG CAGGAACTAG ACTCACCGGC TCAACTTTTG

801 ACAATAATGG AACGCGACAT GCGGCTGATG AACTGCTTAA TAAAGGAGAC

851 CCTAATAACT TGCTAGTTGC AGTTCAGGCC TCAGTAGAGA AGATCCTCTT

901 CTCTTCCAAT ACATCAAgta tgttgcatca gtgatattta atggtagctc

951 ctagtttgtc atgctgcact cgaaaattat tattttatca ttttaaaata

1001 ctaacagaat agtgtgaagt ctcatatttc ccttccatat ttcccaaatt

1051 tccataaaca aaacttccca attctccttc gtttagtttg acaataatta

1101 taagctattc tctaatgcag ATTTGTCAGC TATTGGAGTC ATATATACGG

1151 ATTCTGATGG AAACTCTCAT CAGGCATTTG TACGCGGTAA CGGAGAAGTT

1201 ATTGTTAGTG CAGGGACAAT CGGAACGCCT CAGCTTCTAC TACTTAGTGG

1251 CGTTGGACCA GAGTCTTACC TATCTTCTCT CAACATCACA GTTGTTCAGC

1301 CGAATCCTTA TGTTGGGCAG TTTGTGTATG ACAATCCTCG TAATTTCATT

1351 AATATTTTGC CCCCAAATCC AATTGAAGCC TCTGTTGTAA CTGTTTTAGG

1401 CATTAGAAGT GATTATTATC AAGTTTCTCT GTCAAGCTTG CCATTTTCCA

1451 CTCCACCCTT TAGTCTTTTT CCTACAACAT CTTACCCCCT CCCAAATTCG

1501 ACTTTTGCTC ATATTGTTAG CCAAGTTCCA GGACCATTGT CTCATGGTTC

1551 TGTCACGCTA AATTCATCAT CTGACGTGAG AATCGCTCCA AATATTAAAT

1601 TCAATTACTA TTCAAATTCC ACAGACCTTG CTAATTGTGT TAGCGGCATG

1651 AAGAAGCTTG GTGACTTATT AAGGACAAAG GCATTAGAAC CATATAAAGC

1701 TCGAGATGTG CTGGGAATTG ACGGTTTCAA TTATTTGGGA GTACCTTTGC

1751 CAGAGAACCA AACAGATGAT GCATCCTTCG AAACATTTTG TCTAGATAAT

1801 GTAGCTTCAT ACTGGCATTA CCACGGTGGA AGCCTTGTTG GGAAAGTGCT

1851 TGATGACAGT TTCCGTGTTA TGGGGATCAA AGCATTACGC GTTGTTGATG

1901 CCTCCACTTT CCCTTACGAA CCAAACAGCC ATCCTCAGGG CTTCTATCTG

1951 ATGTTAGGAA Ggtatgtgat gcacacttcc aaccactaga gattctcaat

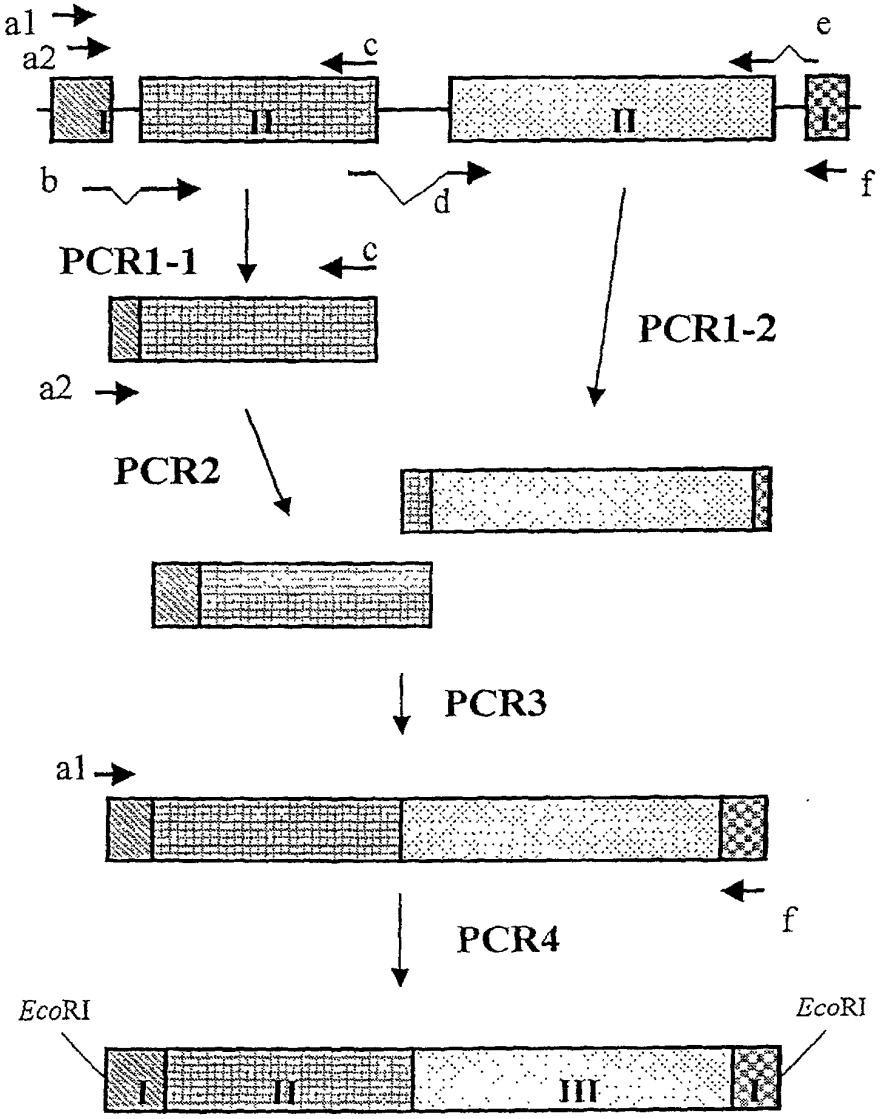
2001 attttgttgt tgttgtaatg aactctctgc cgcattgctc ttttttatta

2051 atccttaaaa ttttgtgttt tgcgcagGTA TGTGGGCCTT CAAATCCTGC

2101 AAGAAAGGTC AATCCGGTTG GAGGCTATTC ATAATATTCA AGAGTCCATG

2151 TGAAGAATTC CG

Figure 2



hnl5 coding region

Figure 3:

Amino acid sequence of the *Prunus amygdalus* hydroxynitrile lyase (HNL5), derived from the nucleotide sequence of the *HNL5* gene. The signal sequence determined from sequence analysis is printed in bold type and the postulated processing site is indicated by an arrow. Possible glycosylation sites (PROSITE patterns) are underlined.

MEKSTMSVILFVLHLLVLHLQYSEVHSLANTSAHDFSYLKFVYNATDTSSEGSYDYI
VIGGGTSGCPLAATLSEKYKVLLLERGTIATEYPNTLTADGFAYNLQQQDDGKTPVE
RFVSEDGIDNVRARILGGTTIINAGVYARANISFYSQTGIEWDLDLVNKTYEWVEDAI
VVKPNNQSWQSVIGEGFLEAGILPDNGFSLDHEAGTRLTGSTFDNNGTRHAADELL
NKGDPNNLLVAVQASVEKILFSSNTSNLSAIGVIYTDSDGNSHQAFVRGNGEVIVSA
GTIGTPQLLLLSGVGPESYLSSLNITVVQPNPYVGQFVYDNPRNFINILPPNPIEASVV
TVLGIRSDYYQVSLSSLPFSTPPFSLFPTTSYPLPNSTFAHIVSQVPGPLSHGSVTLN
SSSDVRIAPNIKFNYYSNSTDLANCVSGMKKLGDLLRTKALEPYKARDVLGIDGFNY
LGVPLPENQTDDASFETFCLDNVASYWHYHGGSLVGKVLDDSFRVMGIKALRVVD
ASTFPYEPNSHPQGFYLMLGRYVGLQILQERSIRLEAIHNIQESM

Figure 4:

Nucleic acid sequence of the DNA fragment coding for a secretory hybrid protein (PamHNL5xGOX) with HNL activity, consisting of sequences of the *Prunus amygdalus HNL5* gene and the *Aspergillus niger* glucose oxidase gene.

gaattcatcatgcagactctccttgtgagctcgcttgtggtctccctcgctgcggccctgcca-
cactacatcaggagcaatggcattgaagcctacaacgccactgatacaagctcggaaggatca-
tatgactacattgtaatcggtggaggaacatcagggtgtccattggcagcaactttatcagaa-
aaatacaaggtgcttcttctagaaagaggcactattgctacagaatacccgaacacgtt-
gactgcagatgggtttgcatataatctgcagcaacaagatgatggaaagacgccagttga-
aaggttcgtgtccgaagatggcattgataatgtgcgagccaggatcctcggtggcacgacca-
taatcaatgcaggcgtctacgccagagctaacatttcattctatagtcaaacaggaatt-
gaatgggacctggatttggtcaataagacatatgagtgggttgaagacgccattgtggt-
caagccaaataatcaatcttggcaatctgttataggagagggattcttggaggcggg-
tattcttccagacaatggatttagtttggatcacgaagcaggaactagactcaccggct-
caacttttgacaataatggaacgcgacatgcggctgatgaactgcttaataaaggagaccc-
taataacttgctagttgcagttcaggcctcagtagagaagatcctcttctcttccaatacat-
caaatttgtcagctattggagtcatatatacggattctgatggaaactctcatcaggcatttg-
tacgcggtaacggagaagttattgttagtgcagggacaatcggaacgcctcagcttctac-
tacttagtggcgttggaccagagtcttacctatcttctctcaacatcacagttgttcagcc-
gaatccttatgttgggcagtttgtgtatgacaatcctcgtaatttcattaatattttgcccc-
caaatccaattgaagcctctgttgtaactgttttaggcattagaagtgattattat-
caagtttctctgtcaagcttgccattttccactccacccctttagtcttttttcctacaacatct-
tacccccctcccaaattcgacttttgctcatattgttagccaagttccaggaccattgtct-
catggttctgtcacgctaaattcatcatctgacgtgagaatcgctccaaatattaaattcaat-
tactattcaaattccacagaccttgctaattgtgttagcggcatgaagaagcttggtgact-
tattaaggacaaaggcattagaaccatataaagctcgagatgtgctgggaattgacggttt-
caattatttgggagtacctttgccagagaaccaaacagatgatgcatccttcgaaa-
cattttgtctagataatgtagcttcatactggcattaccacggtggaagccttgttggga-
aagtgcttgatgacagtttccgtgttatggggatcaaagcattacgcgttgttgatgcctc-
cactttcccttacgaaccaaacagccatcctcagggcttctatctgatgttaggaagg-
tatgtgggccttcaaatcctgcaagaaaggtcaatgcagtgagcggccgcatgcgaattc

Figure 5: Amino acid sequence of the hybrid protein PamHNL5xGOX, derived from the nucleic acid sequence (figure 4).

MQTLLVSSLVVSLAAALPHYIRSNGIEAYNATDTSSEGSYDYIVIGGGTSGCPLAATL
SEKYKVLLLERGTIATEYPNTLTADGFAYNLQQQDDGKTPVERFVSEDGIDNVRARI
LGGTTIINAGVYARANISFYSQTGIEWDLDLVNKTYEWVEDAIVVKPNNQSWQSVIG
EGFLEAGILPDNGFSLDHEAGTRLTGSTFDNNGTRHAADELLNKGDPNNLLVAVQA
SVEKILFSSNTSNLSAIGVIYTDSDGNSHQAFVRGNGEVIVSAGTIGTPQLLLLSGVG
PESYLSSLNITVVQPNPYVGQFVYDNPRNFINILPPNPIEASVVTVLGIRSDYYQVSLS
SLPFSTPPFSLFPTTSYPLPNSTFAHIVSQVPGPLSHGSVTLNSSSDVRIAPNIKFNY
YSNSTDLANCVSGMKKLGDLLRTKALEPYKARDVLGIDGFNYLGVPLPENQTDDAS
FETFCLDNVASYWHYHGGSLVGKVLDDSFRVMGIKALRVVDASTFPYEPNSHPQG
FYLMLGRYVGLQILQERSMQ

Figure 6: Comparison of the amino acid sequences of Prunus amygdalus HNL5 and of the hybrid protein PamHNL5xGOX. Sequence parts of *Aspergillus niger* glucose oxidase are underlined. Sequence regions having no significant homology between the two proteins are printed in italics, and the signal peptides are printed in bold type.

PamHNL5Gox 1 *mqtllvsslv vslaaalphy irsngiea*-- ---------- --YNATDTSS
PamHNL5 1 ***mekstmsvil fvlhllvlhl qysevhslan*** *tsahdfsylk fv*YNATDTSS

PamHNL5Gox 37 EGSYDYIVIG GGTSGCPLAA TLSEKYKVLL LERGTIATEY PNTLTADGFA
PamHNL5 51 EGSYDYIVIG GGTSGCPLAA TLSEKYKVLL LERGTIATEY PNTLTADGFA

PamHNL5Gox 87 YNLQQQDDGK TPVERFVSED GIDNVRARIL GGTTIINAGV YARANISFYS
PamHNL5 101 YNLQQQDDGK TPVERFVSED GIDNVRARIL GGTTIINAGV YARANISFYS

PamHNL5Gox 137 QTGIEWDLDL VNKTYEWVED AIVVKPNNQS WQSVIGEGFL EAGILPDNGF
PamHNL5 151 QTGIEWDLDL VNKTYEWVED AIVVKPNNQS WQSVIGEGFL EAGILPDNGF

PamHNL5Gox 187 SLDHEAGTRL TGSTFDNNGT RHAADELLNK GDPNNLLVAV QASVEKILFS
PamHNL5 201 SLDHEAGTRL TGSTFDNNGT RHAADELLNK GDPNNLLVAV QASVEKILFS

PamHNL5Gox 237 SNTSNLSAIG VIYTDSDGNS HQAFVRGNGE VIVSAGTIGT PQLLLLSGVG
PamHNL5 251 SNTSNLSAIG VIYTDSDGNS HQAFVRGNGE VIVSAGTIGT PQLLLLSGVG

PamHNL5Gox 287 PESYLSSLNI TVVQPNPYVG QFVYDNPRNF INILPPNPIE ASVVTVLGIR
PamHNL5 301 PESYLSSLNI TVVQPNPYVG QFVYDNPRNF INILPPNPIE ASVVTVLGIR

PamHNL5Gox 337 SDYYQVSLSS LPFSTPPFSL FPTTSYPLPN STFAHIVSQV PGPLSHGSVT
PamHNL5 351 SDYYQVSLSS LPFSTPPFSL FPTTSYPLPN STFAHIVSQV PGPLSHGSVT

PamHNL5Gox 387 LNSSSDVRIA PNIKFNYYSN STDLANCVSG MKKLGDLLRT KALEPYKARD
PamHNL5 401 LNSSSDVRIA PNIKFNYYSN STDLANCVSG MKKLGDLLRT KALEPYKARD

PamHNL5Gox 437 VLGIDGFNYL GVPLPENQTD DASFETFCLD NVASYWHYHG GSLVGKVLDD
PamHNL5 451 VLGIDGFNYL GVPLPENQTD DASFETFCLD NVASYWHYHG GSLVGKVLDD

PamHNL5Gox 487 SFRVMGIKAL RVVDASTFPY EPNSHPQGFY LMLGRYVGLQ ILQERS*mg*--
PamHNL5 501 SFRVMGIKAL RVVDASTFPY EPNSHPQGFY LMLGRYVGLQ ILQERS*irle*

PamHNL5Gox 535 --------
PamHNL5 551 *aihniqesm*

Figure 7: Analysis of HNL preparations by SDS PAGE. Details are described in example 11.

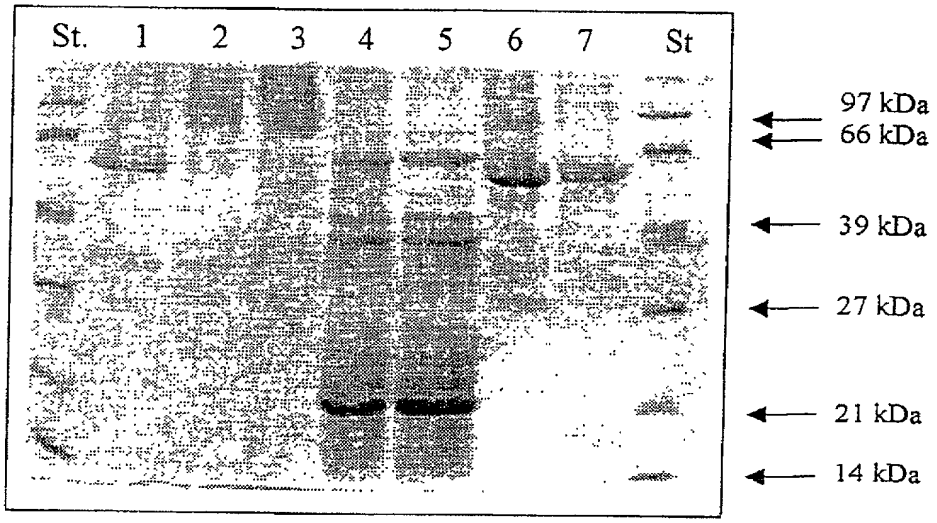


Figure 8:

Nucleotide sequence of the *Prunus amygdalus HNL1* gene obtained by PCR amplification.

ATGGAGAAATCAACAATGTCAGCTATACTGTTGGTGTTATACATTTTTGTCCTCC
ATCTTCAATATTCTGAGGTCCACT
CGCTTGCCACGACTTCTGATCATGgtaaatcacttcaaccgtaattcaaaacaccaaaaagg-
caatcaaaaagaaaacg
gaaaaaagtgtaagaaaagcagatatagacgcctgcatagatgcatgtgctatatactttaaaaactcttcgtctctt
gagattttgcagATTTTAGCTACCTGAGCTTTGCATACGACGCCACTGATCTA-
GAGTTGGAAGGATCATATGACTACGT
TATAGTTGGCGGAGGAACATCAGGGTGTCCATTGGCAGCAACTTTATCAGAAAA
ATACAAGGTGCTCGTTCTCGAAAGG
GGCAGTCTTCCGACAGCATATCCCAACGTCTTGACTGCAGATGGGTTTGTATAT
AATCTCCAGCAAGAAGATGATGGAA
AGACACCGGTCGAAAGGTTCGTGTCCGAAGATGGTATTGATAATGTACGGGGC
AGGGTGCTCGGTGGCACAAGCATTAT
CAATGCCGGTGTCTACGCCAGAGCTAACACCTCAATCTATAGTGCATCAGGAGT
TGATTGGGACATGGATTTGGTTAAT
CAGACATATGAGTGGGTTGAAGACACTATTGTGTACAAGCCAAATTCTCAATCTT
GGCAGTCTGTTACAAAAACTGCAT
TCTTGGAGGCTGGTGTTCATCCAAACCATGGATTTAGTTTAGATCATGAAGAAG
GAACTAGAATTACCGGCTCAACTTT
TGACAACAAGGGAACGAGACATGCAGCTGATGAACTTCTTAATAAAGGAAACTC
TAACAACTTGCGAGTTGGAGTTCAT
GCCTCAGTAGAGAAGATCATCTTCTCCAATGCACCAGgtatgttgcatcatgcactccaa-
aattaatattttgtcattt
taaaacactagcaggagccaaggtctggaagtacgaataaaatttcattattttccttggatttgtttgataatgatta
taagcttttctgtaatgtagGTTTGACAGCTACAGGAGTCATATATAGGGATTCTAATG-
GAACGCCTCACCAAGCATTT
GTACGCAGTAAGGGAGAAGTTATCGTGAGTGCAGGGACAATTGGGACCCCTCA
ACTTCTACTACTTAGCGGTGTTGGGC

CAGAGTCTTACCTATCATCTCTAAATATTCCAGTTGTTCTTTCCCATCCTTACGTC
GGACAGTTTCTGCATGACAATCC
TCGTAATTTCATTAACATTTTGCCCCCAAATCCAATTGAACCCACAATTGTAACTG
YTCTAGGCATTTCAAACGATTTC
TACCAATGTTCTTTCTCGAGCTTGCCATTTACAACTCCACCCTTCGGTTTTTTCC
CTAGTGCATCTTATCCCCTGCCAA
ATTCGACTTTTGCTCACTTTGCTAGCAAAGTGGCAGGACCTTTATCATATGGTTC
TCTCACACTGAAATCATCCTCCAA
TGTGAGAGTCAGTCCAAATGTCAAATTTAATTACTATTCAAATCTGACAGATCTTT
CTCATTGTGTTAGCGGCATGAAG
AAGATTGGTGAACTCTTGAGCACAGACGCATTAAAACCATATAAAGTTGAAGATT
TGCCGGGTGTAGAAGGTTTTAATA
TTTTGGGAATCCCTTTGCCAAAGGACCAAACAGATGATGCAGCCTTCGAAACAT
TTTGCCGAGAATCAGTAGCCTCATA
TTGGCACTACCACGGTGGATGCCTTGTTGGAAAGGTGCTTGATGGTGATTTCCG
TGTTACAGGGATCAATGCATTACGC
GTTGTTGATGGCTCAACATTCCCTTACACACCAGCGAGCCACCCTCAGGGCTTC
TATCTGATGTTAGGGAGgtatgtta
caaattctcaataattatttggttgagtggcttgtttgtaatgaactctatgccatatttctctttctcatcctttcca
tttttgtgccatgggcagGTATGTGGGCATTAAAATTCTGCAAGAAAGATCAGCTTCA-
GATCTAAAAATCTTGGATTCC
CTCAAGTCAGCAGCATCCTTGGTTCTTTAAACT

Figure 9:

Amino acid sequence of *Prunus amygdalus* hydroxynitrile lyase (HNL1), derived from the nucleotide sequence of the *HNL1* gene.

MEKSTMSAILLVLYIFVLHLQYSEVHSLATTSDHDFSYLSFAYDATDLELEGSY
DYVIVGGGTSGCPLAATLSEKYKVLVLERGSLPTAYPNVLTADGFVYNLQQE
DDGKTPVERFVSEDGIDNVRGRVLGGTSIINAGVYARANTSIYSASGVDWDM
DLVNQTYEWVEDTIVYKPNSQSWQSVTKTAFLEAGVHPNHGFSLDHEEGTRI
TGSTFDNKGTRHAADELLNKGNSNNLRVGVHASVEKIIFSNAPGLTATGVIYR
DSNGTPHQAFVRSKGEVIVSAGTIGTPQLLLLSGVGPESYLSSLNIPVVLSHPY
VGQFLHDNPRNFINILPPNPIEPTIVTVLGISNDFYQCSFSSLPFTTPPFGFFPS
ASYPLPNSTFAHFASKVAGPLSYGSLTLKSSSNVRVSPNVKFNYYSNLTDLSHC
VSGMKKIGELLSTDALKPYKVEDLPGVEGFNILGIPLPKDQTDDAAFETFCR
ESVASYWHYHGGCLVGKVLDGDFRVTGINALRVVDGSTFPYTPASHPQGFYL
MLGRYVGIKILQERSASDLKILDSLKSAASLVL